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Molecular Characterization of Circulating Infectious Bursal Disease viruses in chickens from Different Egyptian Governorates during 2023

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Abstract

Infectious bursal disease virus (IBDV) induces severe immunosuppression in chickens, leading to significant economic losses in the global poultry industry. This study investigated 52 chicken flocks, including commercial broilers, layers, and baladi, from various Egyptian governorates for 2023. These flocks exhibited symptoms of depression, along with kidney and bursa lesions, indicative of IBDV infection. Pooled Bursal homogenates were tested using RT-PCR with VP2-specific primers, revealing that 20 flocks tested positive for IBDV. Six representative samples were selected from 20 positive flocks for isolation of embryonated chicken eggs. The embryonic lesions observed included hemorrhage, skull swelling, and liver necrosis with a pale-yellow appearance. Additionally, the harvested chorioallantoic membrane (CAM) showed signs of congestion and thickening. Partial amplification of the VP2 gene from the harvested embryo suspensions of the six IBDV isolates was performed for sequencing. Phylogenetic analysis of the sequences revealed that five IBDV isolates (VV4, VV5, VV6, VV10, and VV16) belonged to the very virulent strain group A3 cluster, whereas one isolate (VV2) clustered with Chinese Variant strains in the A2d group. Sequence analysis of the hypervariable region (HVR) of VP2 compared to that of Egypt-USC-IBD-1-2019 and vvIBDV/Beh21/Egypt/18 highly virulent IBDV strains revealed several amino acid mutations. The VP2 HVR of all isolates maintained the serine-rich heptapeptide sequence SWSASGS, which is adjacent to the major hydrophilic peak B and serves as a virulence marker. Histopathological examination revealed that bursae from chickens infected with very virulent IBDV exhibited marked interlobular edema and lymphoid depletion. In contrast, bursae from chickens infected with Variant IBDV showed massive lymphoid depletion, with hyperplasia of the bursal capsule. These findings highlight the circulation of both virulent and Variant IBDV strains in Egyptian chicken flocks, complicating disease control. Consequently, there is a need to update vaccination programs and vaccine strains for IBDV in Egypt.

1. Introduction

Infectious Bursal disease virus (IBDV) is widely distributed in chickens and is known for its ability to induce severe immunosuppression in infected birds, causing substantial economic losses to the global poultry industry (Dey et al., 2019). It is a highly contagious disease among young chickens and its resilience to various disinfectants and environmental conditions makes it a formidable pathogen. It maintains its infectivity for a period of at least four months in the environment, raising the probability of infection of new flocks that would inhabit the same house in the future. The resistant characteristics of the IBD virus mean that once it contaminates a poultry house, the disease is likely to reappear in future flocks (Zachar et al., 2016).

Its distinctive bi-segmented double-stranded RNA genome and the high error rate associated with its viral RNA-dependent RNA polymerase (RdRp) (von Einem et al., 2004), IBDV is inherently susceptible to Various levels of genetic mutations and recombination events. Notably, there are two serotypes of IBDV, with only serotype I causing disease in chickens, whereas serotype II, which has been isolated from turkeys, does not cause illness in chickens (McFerran et al., 1980). In natural settings, IBDV can infect

chickens of all breeds, and these processes give rise to the emergence and dissemination of new mutant or recombinant strains within chicken populations (Wei et al., 2006).

Multiple primary pathogenic types of IBDV have been recognized, including classical IBDV (cIBDV), Variant IBDV (VarIBDV), very virulent IBDV (vvIBDV), attenuated IBDV, and novel Variant IBDV (nVarIBDV), which vary in terms of their pathogenicity and antigenicity (Zhang et al., 2022). However, there is currently no standardized method for categorizing the IBDV genome, and the classification of IBDV genotypes has become increasingly intricate and imprecise with the emergence of novel strains (Fan et al., 2019). Consequently, conventional classification approaches that rely on pathogenicity and antigenicity require modification. Recently, several enhanced genotyping systems have been proposed, offering substantial assistance in the study of IBDV genetics and molecular epidemiology (WANG et al., 2021). Hence, gaining a comprehensive understanding of the genomic variation patterns of IBDV is essential for the prevention and control of IBD.

The ongoing battle between IBDV and the host takes various forms, with one of the most apparent being viral genomic variation. These genetic changes are crucial for generating diversity and evading the host immune response (Jackwood and Saif, 1987; Kasanga et al., 2007; Van den Berg et al., 2004; Xu et al., 2015). This immunosuppressive virus makes chickens more vulnerable to other pathogens and can even result in mortality (Gao et al., 2023a). Presently, the most common clinical strategy for managing IBD involves vaccinating chickens using inactivated or live attenuated vaccines (Van den Berg et al., 2000). However, the high mutation rate of IBDV is a significant factor behind the emergence of mutant viral strains with antigenic differences from the currently available commercial vaccines (Hou et al., 2022; Snyder et al., 1992; Van den Berg et al., 2004).

The primary objective of our study was to isolate and characterize IBDV strains currently affecting chicken populations across various regions of Egypt. By monitoring the evolutionary dynamics of these strains, we aimed to assess the necessity of revising the existing vaccination programs in Egypt to effectively combat this devastating poultry pathogen.

2. Material and methods

2.1. Sampling

Samples were collected from 52 commercial broilers, layers, and baladi chicken flocks with acute depression, nephritis, or bursal enlargement. During 2023, bursa of Fabricius of diseased and freshly dead chicks from 20 chicken farms in 6 governorates of Egypt (Alexandria, Giza, Beheira, Al-Qalubiya, Al-Mansoura, Al-Gharbia) were collected part kept in freezer for isolation and another part kept in buffered formalin for histopathology. For isolation, the bursae were homogenized with sterile sand and phosphate-buffered saline PBS containing 1000 µl /ml streptomycin and penicillin. Homogenized samples were centrifuged at 3000xg for 10 min. The supernatants were filtered using a 22µl filter, and stored at − 80°C until use (OIE Terrestrial, 2018).

2.2. Virus isolation

Prepared bursal suspensions (0.2 ml) were injected into the chorioallantoic membrane (CAM) of 11–13-day old SPF-ECEs procured from the SPF farm at Koum Oshiem, Fayoum, Egypt. The inoculated eggs were incubated at 37°C for three–five days, then chilled at 4°C overnight. For harvesting, CAM was obtained, washed three times with sterile PBS, and collected in a Falcon tube. Each sample was subsequently subjected to sonication for 1 minute at water bath sonicator (Müller et al., 1979).

2.3. Identification of IBDV using Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

RNA was extracted from the supernatants of the collected CAMs of inoculated SPF-ECEs using the Gene JET Viral DNA/RNA Purification Kit (Thermo Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Two primers were designed in-house to amplify the 800 bp fragment of the IBDV VP2 gene by RT-PCR (forward primer: 5-CTGCAACAGCCAACATCAACG-3 and reverse primer: 5-CAAGACGGTCCCTCTCACT-3). cDNA was prepared using the Maxima H Minus Double-Stranded cDNA Synthesis Kit (Thermo Scientific, Waltham, MA, USA), according to the manufacturer's instructions. PCR thermal cycler (StepOnePlus™ Real-Time PCR System, Applied Biosystems, Waltham, MA, USA) was used with the following cycling program: 95°C for 5 min; 35 three-step cycles of 94°C for 10s (denaturation), 52°C for 20s (annealing), and 72°C for 50s; then 72°C for 10 min (final extension). PCR products were electrophoresed against GeneRuler 100 bp Plus DNA Ladder (Thermo Scientific™, Waltham,) on a 1.5% agarose gel containing ethidium bromide at a final concentration of 0.5 µg/ml at 120V for 30 min. in 1x TBE buffer (Gao et al., 2023b).

2.4. Sequence analysis of VP2 of IBDV

The purified PCR products were sequenced with the ABI PRISM ® Big Dye TM Terminators v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and an ABI PRISM® 3130 genetic analyzer with 80 cm capillaries (Applied Biosystems). The sequences were edited in SeqScape Software Version 2.5 (Applied Biosystems), and consensus sequence assembly and alignment trimming were performed using the Lasergene DNASTAR suite of programs (DNASTAR Inc., Madison, WI, USA) using the Clustal W method. According to (Tamura et al., 2021), sequence alignment, identity, divergence, and phylogram were generated using the DNAstar-MegAlign program. Egyptian IBD viruses and other international reference strains were retrieved from NCBI, as were infectious bursal disease viruses from the National Centre for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov).

2.5. Histopathology

Bursas were collected from 3 to 5 birds from each flock and fixed in 10% neutral buffered formalin. The tissues were processed, and 4µm-thick sections were cut from the paraffin-embedded tissue blocks. These sections were stained with hematoxylin and eosin, as described by (Suvarna et al., 2018), for routine histopathological (HP) examination under a light microscope.

3. Results

3.1. Farm History and Mortality Rates of IBDV-Affected Chicken Flocks

Chickens from the examined farms displayed varying degrees of hemorrhage in their breast and thigh muscles, along with swollen and hemorrhagic bursa. The mortality rates in the examined chicken flock ranged from 3–20%, calculated over a 7-day period following the onset of increased mortality. The results indicated that both the type and timing of vaccination, as well as the age and type of chicken, significantly influenced the severity of IBDV infection and associated mortality rates. Mortality rates ranged from 2–20%, with the highest mortality observed in flock VV7 (20%), and the lowest in flocks VV9 and VV8 (3%). The severity of the lesions ranged from mild (+) to severe (+++), with the most severe lesions observed in flocks VV5, VV10, and VV11. Moreover, the type and timing of vaccinations varied across flocks, with different vaccines, such as Transmmune, Vaxxitek, D78, Winterfield 228, IBDL (Ceva), Bursin Plus, Innovax, Bursaplex, and Intermediate, used at different ages (Table 1).

Table 1
Farm History Mortality rates for IBDV including age, vaccination program, severity of PM lesions, and location

Flock Code	Chicken type	Governate	Age (days)	Flock size	IBDV vaccination		Mortality	Severity
					Age	Vaccine		
VV1	Broiler	Alexandria	24	5000	1	Transmmune	10%	++
					8	Intermediate		
VV2	Broiler	Giza	24	20000	1	vaxxitek	6%	+
VV3	Broiler	Beheira	30	110000	1	vaxxitek	3%	++
					8	D78		
VV4	Layers	Al-Qalyubia	22	8000	13	Winterfield 228	6%	++
VV5	Layers	Al-Qalyubia	39	4600	14	IBDL (ceva)	6%	+++
VV6	Broiler	Giza	31	149500	1	vaxxitek	3%	++
					13	Intermediate		
VV7	Broiler	Al-Qalyubia	30	5000	14	Bursin plus	20%	++
VV8	Broiler	Al-Qalyubia	34	10500	1	Innovax	3%	++
					15	D78		
VV9	Broiler	Al-Qalyubia	24	11000	1	Bursaplex	2%	+
					12	Intermediate		
VV10	Layers	Al-Qalyubia	42	4500	7	inactivated	12%	+++
					12	IBDL ceva		
VV11	Layers	Al-Qalyubia	30	24000	8	inactivated	8%	+++
					12	IBDL		
					19	Winterfield 228		
VV12	Broiler	Alexandria	30	8000	12	Intermediate	8%	++
VV13	Broiler	Al-Qalyubia	32	7000	1	Transmmune	6%	++
VV14	Baladi	Al-	36	10000	12	Intermediate	7%	+

* Severity: +indicates mild, ++ indicates moderate, +++ indicates severe

Flock Code	Chicken type	Governate	Age (days)	Flock size	IBDV vaccination		Mortality	Severity
					Age	Vaccine		
		Mansoura				plus		
VV15	Broiler	Al-Mansoura	28	9000	1 8	vaxxitek Intermediate	4%	++
VV16	Baladi	Al-Gharbia	30	10000	12	Intermediate plus	6%	++
VV17	Baladi	Al-Mansoura	25	7000	12	Intermediate plus		++
VV18	Broiler	Al-Qalyubia	25	3500	12	hot	8%	++
VV19	Broiler	Giza	24	20000	1	vaxxitek	5%	+
VV20	Broiler	Giza	24	20000	1	vaxxitek	9%	+
* Severity: +indicates mild, ++ indicates moderate, +++ indicates severe								

3.2. Molecular screening of IBDV using RT-PCR

Molecular screening of 52 chicken farms using RT-PCR with VP2-specific primers revealed that 20 farms (38.4%) tested positive for Infectious Bursal Disease Virus (IBDV).

3.3. IBDV isolation

Six samples that showed positive RT-PCR bands were inoculated into SPF-ECE via the chorioallantoic membrane (CAM) route at 11-day-old. Embryos showed signs suggestive of IBDV replication compared with the negative control. These signs included hemorrhage, skull swelling, and liver necrosis, which appeared pale yellow in color. Furthermore, the harvested CAMs were congested and thick (Fig. 1). CAM and liver from inoculated embryos were collected and processed for VP2 amplification by RT-PCR with successful amplification of 800 base pair bands (Fig. 2) that purified and sent for sequencing

3.4. Sequence analysis of the hyper Variable region of VP2

RT-PCR analysis confirmed the presence of Infectious Bursal Disease Virus (IBDV) in six isolates. A 743-bp nucleotide sequence encoding 247 amino acids encompassing the region flanking the Hypervariable Region (HVR) of VP2 was characterized. For detailed analysis, a 420-bp segment encoding 140 amino acids within the VP2 HVR was examined (Fig. 3). All the identified and sequenced IBDV strains spanned nucleotide positions 625–1,044 and amino acids 211–350. Notable amino acid mutations were also observed in these isolates. VV5 exhibited S299N, VV10, VV4 showed Q221K and A222T, and VV16 had A270E. VV6 did not show any mutation. The VV2 isolate demonstrated multiple mutations (V252I, S254N, I256V, D279N, T286I, I294L, G318D, A321V, and D323E), indicating its relationship with the

Chinese Variant strains. Despite these variations, all isolates retained the serine-rich heptapeptide sequence SWSASGS, a key virulence marker adjacent to major hydrophilic peak B.

The identity matrix revealed that five isolates (VV4, VV5, VV6, VV10, and VV16) shared 94–100% identity with the A3 group reference strains, including Egypt-USC-IBD-1-2019, HuB-1, HK46, and 58/92. In contrast, the VV2 isolate showed 97% identity to the Chinese Variant, suggesting a close relationship with ZD-2018-1. All six isolates showed 90–94% identity with vaccine strain 57/70 in the A1 group (Fig. 4). (Table 2) contain a comprehensive illustration of amino acid mutations in VP2 compared to other isolates, as per the findings of a recent study (Gao et al., 2023a).

Table 2 Amino acids mutation of VP2 for IBDv compared with isolates according to (Gao et al., 2023a)

aa	Function	vv4	vv5	vv6	vv10	vv16	vv2	IBD-1	MK496544.1 Beh21/Egypt/18	AF498632.1 Bursine Plus	EU544157.1 Cevac-IBD-L	MN485882.1 ZD-2018-1
213	Immune escape	D	D	D	D	D		D	D	D	D	N
219	Virus assembly	Q	Q	Q	Q	Q		Q	Q	Q	Q	Q
221	Immune escape	K	Q	Q	K	Q		Q	Q	Q	Q	K
222	Immune escape; Virus replication and virulence-related	T	A	A	T	A		A	A	L	P	T
223	Immune escape	G	G	G	G	G		G	G	G	G	G
234-236	Intermolecular interactions	IDA	IDA	IDA	IDA	IDA		IDA	IDA	IDA	IDA	IDA
249	Immune escape; Virus replication and virulence-related	Q	Q	Q	Q	Q	Q	Q	Q	H	Q	K
253	Cellular adaptability; virulence-related	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q
254	Immune escape	S	S	S	S	S	N	S	S	G	G	N
256	Virus replication and virulence-related	I	I	I	I	I	V	I	I	A	V	V
270	attenuation	A	A	A	A	E	A	A	A	T	T	A
279	Cellular adaptability	D	D	D	D	D	N	D	D	D	D	N
284	Cellular adaptability	A	A	A	A	A	A	A	A	T	A	A
286	Immune escape	T	T	T	T	T	S	T	T	I	T	I
299	Cellular adaptability	S	N	S	S	S	E	S	S	N	N	S
318	Immune escape	G	G	G	G	G	D	G	G	G	G	D
321	Immune escape; virulence-related	A	A	A	A	A	V	A	A	A	A	A
323	Immune escape	D	D	D	D	D	E	D	D	D	D	E
324	Immune escape; virus assembly	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q

IBD-1: MT304668.1 Egypt-USC-IBD-1-2019

3.5. Phylogenetic Analyses for HVR of VP2

A phylogenetic tree constructed from the aligned HVR sequences revealed the formation of nine major clusters corresponding to the proposed genogroups. Among these, five isolates, EGY/IBDV/Qalubiya/VV4/2023,EGY/IBDV/Qalubiya/VV5/2023,EGY/IBDV/Qalubiya/VV10/2023, EGY/IBDV/El-Gharbia/VV16/2023, and EGY/IBDV/GIZA/VV6/2023, clustered within the A3 group of very

virulent IBDV strains, as classified by (WANG et al., 2021). This A3 cluster included strains commonly found globally, such as HuB-1 from Europe, HK46 from Asia, and Egypt-USC-IBD-1-2019 from the Middle East (Fig. 5). Notably, the isolate EGY/IBDV/GIZA/VV2/2023 was distinct, clustering with Chinese Variant strains within the A2d group.

3.5. Histopathology

Histopathological analysis of bursae from chickens infected with very virulent IBDV (vvIBDV) demonstrated severe pathological changes. Specifically, the bursae exhibited marked interlobular edema, characterized by swelling between lobules and substantial lymphoid depletion, indicating a significant loss of lymphoid tissue. Lymphoid depletion disrupts the normal structure and function of the bursa, which is crucial for immune development in chickens. In contrast, the bursae from chickens infected with Variant IBDV showed a different pattern of pathology. Massive lymphoid depletion similar to that observed in vvIBDV infections was observed; however, this was accompanied by hyperplasia of the bursal capsule. Hyperplasia refers to an abnormal increase in the number of cells within the bursal capsule, which results in the thickening and enlargement of the capsule. (Fig. 6).

4. Discussion

In Egypt, as in other major poultry-producing countries, the Infectious Bursal Disease Virus (IBDV) poses a significant threat to poultry health and productivity. Effective strategies are crucial to prevent the spread of IBDV and mitigate its impact. Recent advances in reverse genetics offer promising tools for developing new vaccines and for understanding viral pathogenesis (Metwally et al., 2003; Mundt et al., 2003; Yang and Ye, 2020). This study identified and characterized IBDV strains using RT-PCR, a highly sensitive method for detecting the virus (Müller et al., 2003). Of the 52 field samples tested, 20 tested positive for IBDV. Post-mortem examinations revealed a range of mild to severe lesions, including congestion and hemorrhage in the thigh muscles and edematous, hemorrhagic bursae. The observed clinical signs vary based on factors such as viral virulence, bird age, and immune status (Rauw et al., 2007). Highly virulent strains are known to cause more severe pathogenesis than less virulent ones (Berg, 2000) .

The study also noted that IBDV strains isolated from vaccinated broiler flocks exhibited signs of IBD despite vaccination, highlighting the capacity of the virus to induce immunosuppression even in previously vaccinated birds. This aligns with findings that Variant IBDV strains can replicate and cause significant damage, even in the presence of immunity to classical strains (Fan et al., 2020). Previous studies have linked antigenic variation in IBDV to amino acid substitutions within the Hypervariable Region (HVR) of the capsid protein, which is crucial for understanding shifts in antigenicity (Heine et al., 1991; Jackwood et al., 1997; Liu et al., 1994). Specific amino acid changes, including those in 213N, 222T, 249 K, 254S, 270A, 286I, 318D, and 323E, have been documented in Variant E strains. Additionally, S254N substitution has been observed in the Chinese SHG13 strain (Fan et al., 2019). However, not all Variant strains exhibit the same amino acid substitutions, emphasizing the complexity of antigenic variation (Jackwood and Sommer-Wagner, 2011). Our study focused on the hypervariable region (HVR)

of the VP2 protein of Infectious Bursal Disease Virus (IBDV), specifically spanning amino acids 206–350. This region, known for its role in strain diversity, was selected for sequence analysis to characterize IBDV strains. Examination of this region revealed significant genetic variation among the IBDV isolates from Egypt and China. The analysis identified specific amino acid mutations in the isolates, such as A270E in EGY/IBDV/El-Gharbia/VV16/2023/VP2 and S299N in EGY/IBDV/Qalubiya/VV5/2023/VP2, and a range of mutations, including G254N, I256V, D279N, T286I, G318D, A321V, and D323E in EGY/IBDV/GIZA/VV2/2023/VP2, as well as Q221K and A222T in EGY/IBDV/Qalubiya/VV4/2023/VP2.

The identified mutations were situated within the VP2 hypervariable region, which is critical for antigenicity and immune evasion. These findings suggest that these mutations may affect the ability of the virus to evade the neutralizing antibodies generated by vaccination. This phenomenon, known as antigenic drift, is driven by changes in specific residues, such as 222T, 249 K, 254S, 286I, and 318D. Mutations at these sites are associated with alterations in antigenicity, which can lead to vaccine escape (Jackwood and Sommer-Wagner, 2011; Letzel et al., 2007).

Moreover, our analysis revealed that mutations like 256V, which enhances replication in vitro, may actually reduce replication in vivo. This underscores the complex relationship between antigenic variations and viral fitness. Specifically, while mutations such as A321V in isolate 94432 were linked to reduced pathogenicity and replication, persistent antigenically atypical strains in Egypt indicated that these mutations might confer advantages under selective immune pressures. This suggests that the evolutionary dynamics of IBDV are influenced by both the host's immune response and vaccination strategies (Domanska et al., 2004; Escaffre et al., 2013).

Recent findings have also highlighted mutations at residues 318 and 323, which have been shown to impact neutralization by antiserum, thereby emphasizing their role in antigenic variation (Fan et al., 2022). This indicates that these mutations may allow IBDV strains to infect chicks, even in the presence of high levels of maternal antibodies, thus complicating control efforts. Histopathological analysis of bursa samples from chickens infected with different strains of Infectious Bursal Disease Virus (IBDV) revealed distinct patterns of tissue damage, reflecting the varying pathogenic mechanisms of these strains. Chickens infected with virulent IBDV (vvIBDV) exhibit severe interlobular edema and significant lymphoid depletion within the bursa. The presence of pronounced interlobular edema indicates severe inflammation and swelling between the bursal lobules, which, combined with substantial lymphoid depletion, suggests profound disruption of the bursa's immune architecture. This extensive lymphoid loss severely impaired the ability of the bursa to support effective immune responses, underscoring the debilitating impact of vvIBDV on the immune system.

In contrast, chickens infected with Variant IBDV demonstrated a different histopathological profile. These birds showed massive lymphoid depletion along with hyperplasia of the bursal capsule. Hyperplasia of the bursal capsule, characterized by an increase in the number of cells within this structure, suggests a compensatory mechanism in response to extensive loss of lymphoid tissue. This adaptation may influence the progression and severity of the disease as the bursal capsule attempts to counterbalance

the diminished lymphoid mass. The coexistence of massive lymphoid depletion with capsule hyperplasia indicates that Variant IBDV strains may employ different mechanisms to impact the bursa than vvIBDV strains.

These findings align with recent observations in Egyptian chicken flocks in 2023, as reported by (Salaheldin et al., 2024), further validating the distinct pathological outcomes associated with different IBDV strains. The observed histopathological changes not only reflect the differential pathogenic mechanisms of vvIBDV and Variant IBDV but also underscore the need for tailored approaches to manage and control IBD outbreaks. Understanding these distinct tissue damage patterns is crucial for developing effective strategies to mitigate the effects of various IBDV strains on poultry health.

5. Conclusion

This study successfully characterized five very virulent IBDV (vvIBDV) strains and one Chinese Variant strain from field cases of infectious bursal disease (IBD) in Egypt in 2023. Genotyping revealed that these strains exhibited progressive evolution and persisted in an Egyptian environment, indicating an ongoing challenge with vvIBDV in the region. Our analysis demonstrated significant genetic diversity and pathogenic variability among IBDV strains in Egyptian chicken flocks. Molecular characterization of the VP2 hypervariable region (HVR) revealed critical genetic differences among the strains, impacting vaccine efficacy and disease management strategies. Additionally, the distinct histopathological profiles associated with different IBDV strains highlighted the need for tailored vaccination and control measures, which require continuous surveillance of circulating strains, regular updates to vaccination programs, and targeted control measures based on the characteristics of the prevalent strains. The identification of specific mutations within the VP2 HVR emphasized the virus's ongoing evolution, affecting its antigenicity and pathogenicity. In conclusion, the study's findings are pivotal for guiding vaccine development and understanding IBDV's adaptive mechanisms. The persistence of antigenically atypical strains in regions such as Egypt underscores the need for continuous monitoring and adaptation of vaccination strategies. Future research should focus on the evolutionary pressures driving these mutations and their impact on vaccine efficacy and viral control, to ensure effective strategies against evolving IBDV strains.

Declarations

Ethical approval

Ethical approval for this study was given by the Institutional Animal Care and Use Committee under University of Sadat City No. ……..

Ethical consideration

The authors confirm that all authors have reviewed and submitted the manuscript to this journal for the first time.

Conflict of interests

The authors have not declared any conflict of interest.

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Author Contribution

A.H.A. and D.A. conceptualized the study and designed the experiments.A.H.A.,M.S. and S.E.M.E. conducted the molecular and histopathological analyses.A.H.A.,A.A.E. and M.I. performed the data analysis and interpretation.A.H.A.,M.K. and A.A. prepared the figures and tables.A.H.A., D.A., M.S., S.E.M.E., A.A.E., and M.I. wrote the main manuscript text.All authors reviewed and approved the final manuscript.

Availability of data and materials

The original contributions presented in the study are included in the article/supplementary material. For inquiries, please contact the corresponding author/s.

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Figures

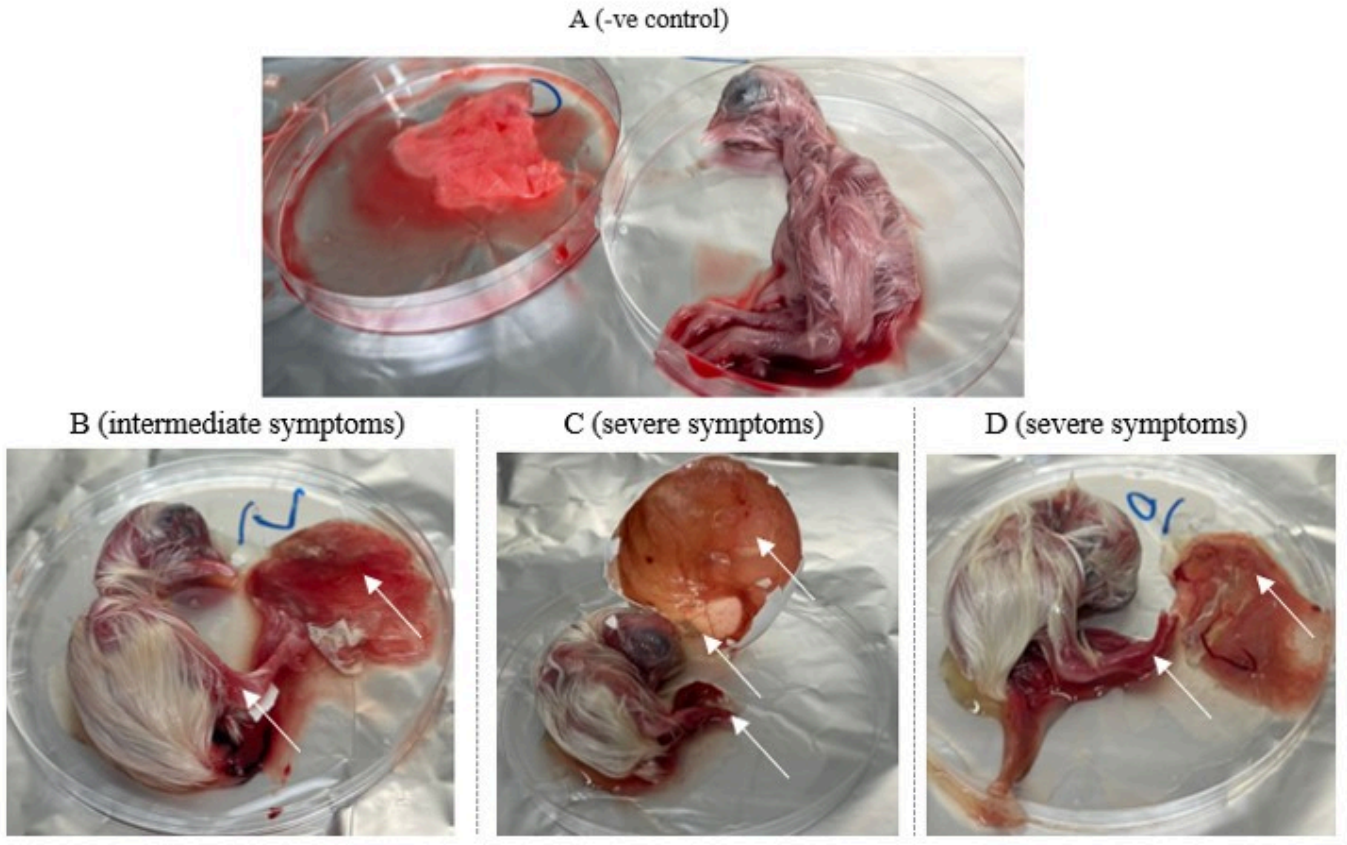


Figure 1

The harvested egg embryos showed hemorrhage, edema of the skull, harvested CAM became congested and thickened A negative control, B exhibited Intermediate symptoms on the embryo, C and D severe symptoms on the embryo

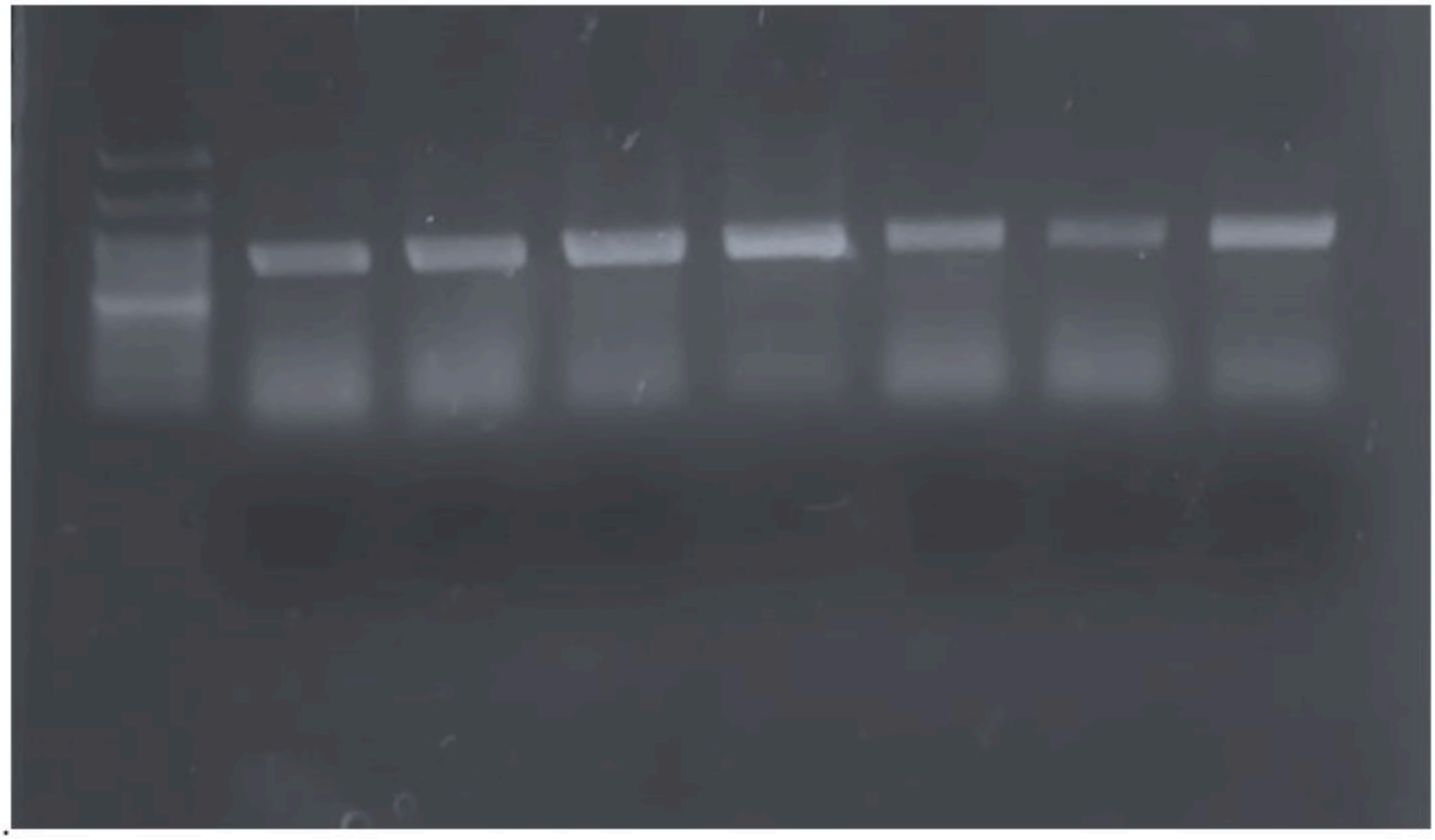


Figure 2

Gel electrophoresis for amplified VP2 bands by RT-PCR, the expected band size (800 bp). Lane 1 +ve control, Lanes 2-7 for six IBDV isolates.

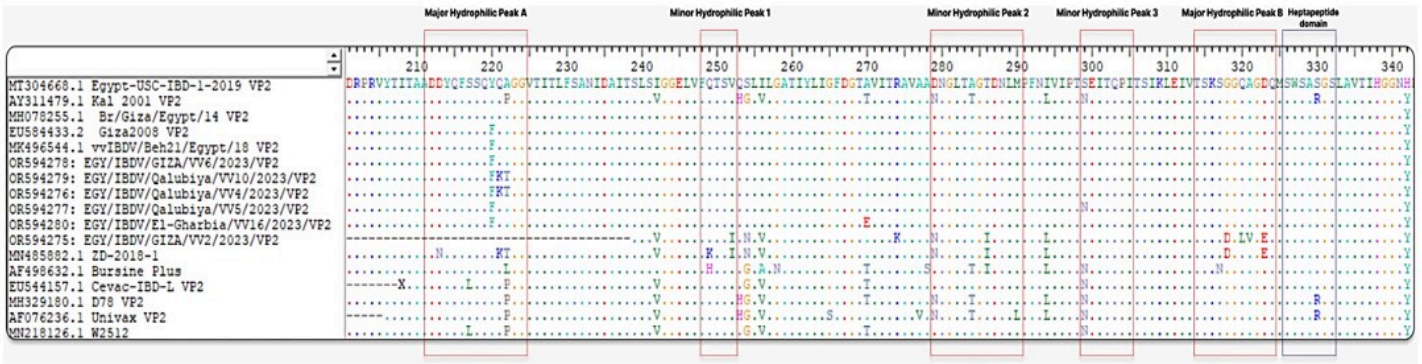
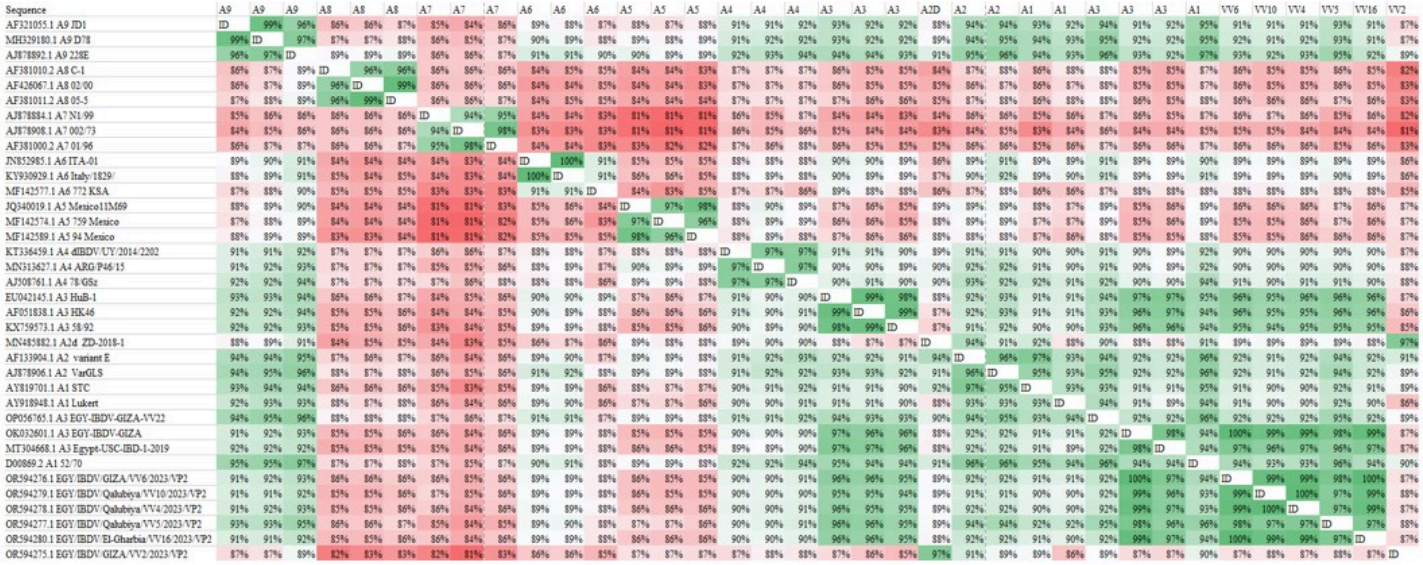


Figure 3

Deduced amino acid alignment of the VP2 hypervariable region from aa position 211 to 350 of IBDV strains. Dots indicate aa positions. Major and minor hydrophilic peaks are boxed with a red line.



Sequence	A9	A9	A9	A8	A8	A8	A7	A7	A7	A6	A6	A6	A5	A5	A5	A4	A4	A4	A3	A3	A3	A2D	A2	A2	A1	A1	A3	A3	A3	A1	VV6	VV10	VV4	VV5	VV16	VV2
AF321055.1 A9 JD1	ID	99%	96%	86%	86%	87%	85%	84%	86%	89%	88%	87%	88%	87%	88%	91%	91%	92%	93%	92%	92%	88%	94%	94%	93%	92%	94%	91%	92%	95%	91%	91%	91%	93%	91%	87%
MH329180.1 A9 D78	99%	ID	97%	87%	87%	88%	86%	85%	87%	90%	89%	88%	89%	88%	89%	91%	92%	92%	93%	92%	92%	89%	94%	95%	94%	93%	95%	92%	92%	95%	92%	91%	92%	93%	91%	87%
AJ878892.1 A9 228E	96%	97%	ID	89%	89%	89%	86%	86%	87%	91%	91%	90%	90%	89%	89%	92%	93%	94%	94%	94%	93%	91%	95%	96%	94%	93%	96%	93%	92%	97%	93%	92%	93%	95%	92%	89%
AF381010.2 A8 C-1	86%	87%	89%	ID	96%	96%	86%	86%	86%	84%	85%	85%	84%	84%	83%	87%	87%	87%	86%	85%	85%	84%	87%	88%	86%	88%	88%	85%	85%	87%	86%	85%	85%	86%	85%	82%
AF426067.1 A8 02/00	86%	87%	89%	96%	ID	99%	86%	86%	86%	84%	84%	85%	84%	84%	83%	87%	87%	87%	86%	85%	85%	85%	86%	87%	86%	87%	88%	85%	85%	87%	86%	85%	85%	86%	85%	83%
AF381011.2 A8 05-5	87%	88%	89%	96%	99%	ID	86%	86%	87%	84%	85%	85%	84%	84%	84%	87%	87%	87%	87%	86%	86%	85%	87%	88%	86%	88%	88%	86%	85%	88%	86%	86%	86%	87%	86%	83%
AJ878884.1 A7 N1/99	85%	86%	86%	86%	86%	86%	ID	94%	95%	84%	84%	83%	81%	81%	81%	86%	85%	87%	84%	84%	83%	84%	86%	86%	85%	86%	87%	86%	85%	87%	86%	87%	86%	86%	85%	82%
AJ878908.1 A7 002/73	84%	85%	86%	86%	86%	86%	94%	ID	98%	83%	83%	83%	81%	81%	81%	86%	85%	86%	85%	84%	84%	83%	84%	85%	83%	84%	86%	84%	84%	85%	85%	85%	84%	84%	84%	81%
AF381000.2 A7 01/96	86%	87%	87%	86%	86%	87%	95%	98%	ID	84%	84%	83%	83%	82%	82%	87%	86%	88%	86%	85%	85%	85%	86%	86%	85%	86%	87%	86%	86%	87%	86%	86%	86%	86%	85%	83%
JN852985.1 A6 ITA-01	89%	90%	91%	84%	84%	84%	84%	83%	84%	ID	100%	91%	85%	85%	85%	88%	88%	88%	90%	90%	89%	86%	89%	91%	89%	89%	91%	89%	89%	90%	89%	89%	89%	89%	89%	86%
KY930929.1 A6 Italy/1829/	88%	89%	91%	85%	84%	85%	84%	83%	84%	100%	ID	91%	86%	86%	85%	88%	89%	88%	90%	89%	89%	87%	90%	92%	89%	90%	91%	89%	89%	91%	89%	89%	89%	90%	89%	86%
MF142577.1 A6 772 KSA	87%	88%	90%	85%	85%	85%	83%	83%	83%	91%	91%	ID	84%	83%	85%	87%	87%	86%	89%	88%	88%	86%	87%	88%	86%	86%	87%	88%	88%	88%	88%	88%	88%	88%	88%	85%
JQ340019.1 A5 Mexico11M69	88%	89%	90%	84%	84%	84%	81%	81%	83%	85%	86%	84%	ID	97%	98%	88%	90%	89%	87%	86%	85%	89%	89%	89%	88%	87%	89%	85%	86%	89%	86%	86%	86%	87%	86%	87%
MF142574.1 A5 759 Mexico	87%	88%	89%	84%	84%	84%	81%	81%	82%	85%	86%	83%	97%	ID	96%	88%	89%	89%	86%	86%	85%	88%	89%	89%	87%	87%	89%	85%	86%	89%	85%	85%	86%	87%	86%	87%
MF142589.1 A5 94 Mexico	88%	89%	89%	83%	83%	84%	81%	81%	82%	85%	85%	85%	98%	96%	ID	88%	89%	88%	87%	86%	86%	88%	88%	88%	87%	86%	88%	85%	85%	88%	85%	85%	86%	86%	86%	87%
KT336459.1 A4 dIBDV/UY/2014/2202	91%	91%	92%	87%	87%	87%	86%	86%	87%	88%	88%	87%	88%	88%	88%	ID	97%	97%	91%	91%	90%	89%	91%	91%	90%	90%	91%	90%	89%	92%	90%	90%	90%	90%	90%	87%
MN313627.1 A4 ARG P46/15	91%	92%	93%	87%	87%	87%	85%	85%	86%	88%	89%	87%	90%	89%	89%	97%	ID	97%	90%	90%	89%	90%	92%	92%	91%	90%	91%	90%	89%	92%	90%	90%	90%	90%	90%	88%
AJ508761.1 A4 78/GSz	92%	92%	94%	87%	87%	87%	87%	86%	88%	88%	88%	86%	89%	89%	88%	97%	97%	ID	90%	91%	90%	90%	93%	92%	92%	91%	92%	90%	90%	94%	91%	90%	91%	91%	90%	88%
EU042145.1 A3 HuB-1	93%	93%	94%	86%	86%	87%	84%	85%	86%	90%	90%	89%	87%	86%	87%	91%	90%	90%	ID	99%	98%	88%	92%	93%	91%	91%	94%	97%	97%	95%	96%	95%	96%	96%	96%	87%
AF051838.1 A3 HK46	92%	92%	94%	85%	85%	86%	84%	84%	85%	90%	89%	88%	86%	86%	86%	91%	90%	91%	99%	ID	99%	87%	92%	93%	91%	91%	93%	96%	97%	94%	96%	95%	95%	96%	96%	86%
KX759573.1 A3 58/92	92%	92%	93%	85%	85%	86%	83%	84%	85%	89%	89%	88%	85%	85%	86%	90%	89%	90%	98%	99%	ID	87%	91%	92%	90%	90%	93%	96%	96%	94%	95%	94%	95%	95%	95%	85%
MN485882.1 A2d ZD-2018-1	88%	89%	91%	84%	85%	85%	84%	83%	85%	86%	87%	86%	89%	88%	88%	89%	90%	90%	88%	87%	87%	ID	94%	91%	92%	88%	90%	88%	88%	91%	89%	89%	89%	89%	88%	97%
AF133904.1 A2 variant E	94%	94%	95%	87%	86%	87%	86%	84%	86%	89%	90%	87%	89%	89%	88%	91%	92%	93%	92%	92%	91%	94%	ID	96%	97%	93%	94%	92%	92%	96%	92%	91%	92%	94%	92%	91%
AJ878906.1 A2 VarGLS	94%	95%	96%	88%	87%	88%	86%	85%	86%	91%	92%	88%	89%	89%	88%	91%	92%	92%	93%	93%	92%	91%	96%	ID	95%	93%	95%	92%	92%	96%	92%	91%	92%	94%	92%	89%
AY819701.1 A1 STC	93%	94%	94%	86%	86%	86%	85%	83%	85%	89%	89%	86%	88%	87%	87%	90%	91%	92%	91%	91%	90%	92%	97%	95%	ID	93%	93%	91%	91%	95%	91%	90%	90%	92%	91%	89%
AY918948.1 A1 Lukert	92%	93%	93%	88%	87%	88%	86%	84%	86%	89%	90%	86%	87%	87%	86%	90%	90%	91%	91%	91%	90%	88%	93%	93%	93%	ID	94%	91%	89%	94%	91%	90%	90%	92%	90%	86%
OP056765.1 A3 EGY-IBDV-GIZA-VV22	94%	95%	96%	88%	88%	88%	87%	86%	87%	91%	91%	87%	89%	89%	88%	91%	91%	92%	94%	93%	93%	90%	94%	95%	93%	94%	ID	92%	92%	96%	92%	92%	92%	95%	92%	89%
OK032601.1 A3 EGY-IBDV-GIZA	91%	92%	93%	85%	85%	86%	86%	84%	86%	89%	89%	88%	85%	85%	85%	90%	90%	90%	97%	96%	96%	88%	92%	92%	91%	91%	92%	ID	98%	94%	100%	99%	99%	98%	99%	87%
MT304668.1 A3 Egypt-USC-IBD-1-2019	92%	92%	92%	85%	85%	85%	85%	84%	86%	89%	89%	88%	86%	86%	85%	89%	89%	90%	97%	97%	96%	88%	92%	92%	91%	89%	92%	98%	ID	94%	97%	96%	97%	96%	97%	87%
D00869.2 A1 52/70	95%	95%	97%	87%	87%	88%	87%	85%	87%	90%	91%	88%	89%	89%	88%	92%	92%	94%	95%	94%	94%	91%	96%	96%	95%	94%	96%	94%	94%	ID	94%	93%	93%	96%	94%	90%
OR594276.1 EGY/IBDV/GIZA/VV6/2023/VP2	91%	92%	93%	86%	86%	86%	86%	85%	86%	89%	89%	88%	86%	85%	85%	90%	90%	91%	96%	96%	95%	89%	92%	92%	91%	91%	92%	100%	97%	94%	ID	99%	99%	98%	100%	87%
OR594279.1 EGY/IBDV/Qalubiya/VV10/2023/VP2	91%	91%	92%	85%	85%	86%	87%	85%	86%	89%	89%	88%	86%	85%	85%	90%	90%	90%	95%	95%	94%	89%	91%	91%	90%	90%	92%	99%	96%	93%	99%	ID	100%	97%	99%	88%
OR594278.1 EGY/IBDV/Qalubiya/VV4/2023/VP2	91%	92%	93%	85%	85%	86%	86%	84%	86%	89%	89%	88%	86%	86%	86%	90%	90%	91%	96%	95%	95%	89%	92%	92%	90%	90%	92%	99%	97%	93%	99%	100%	ID	97%	99%	87%
OR594277.1 EGY/IBDV/Qalubiya/VV5/2023/VP2	93%	93%	95%	86%	86%	87%	85%	84%	85%	89%	90%	88%	87%	87%	86%	90%	90%	91%	96%	96%	95%	89%	94%	94%	92%	92%	95%	98%	96%	96%	98%	97%	97%	ID	97%	88%
OR594280.1 EGY/IBDV/El-Gharbia/VV16/2023/VP2	91%	91%	92%	85%	85%	86%	86%	84%	86%	89%	89%	88%	86%	86%	86%	90%	90%	90%	96%	96%	95%	88%	92%	92%	91%	90%	92%	99%	97%	94%	100%	99%	99%	97%	ID	87%
OR594275.1 EGY/IBDV/GIZA/VV2/2023/VP2	87%	87%	89%	82%	83%	83%	82%	81%	83%	86%	86%	85%	87%	87%	87%	87%	88%	88%	87%	86%	85%	97%	91%	89%	89%	86%	89%	87%	87%	90%	87%	88%	87%	88%	87%	ID

Figure 4

Identity Matrix between IBDV isolates and other Egyptian and representative reference strains

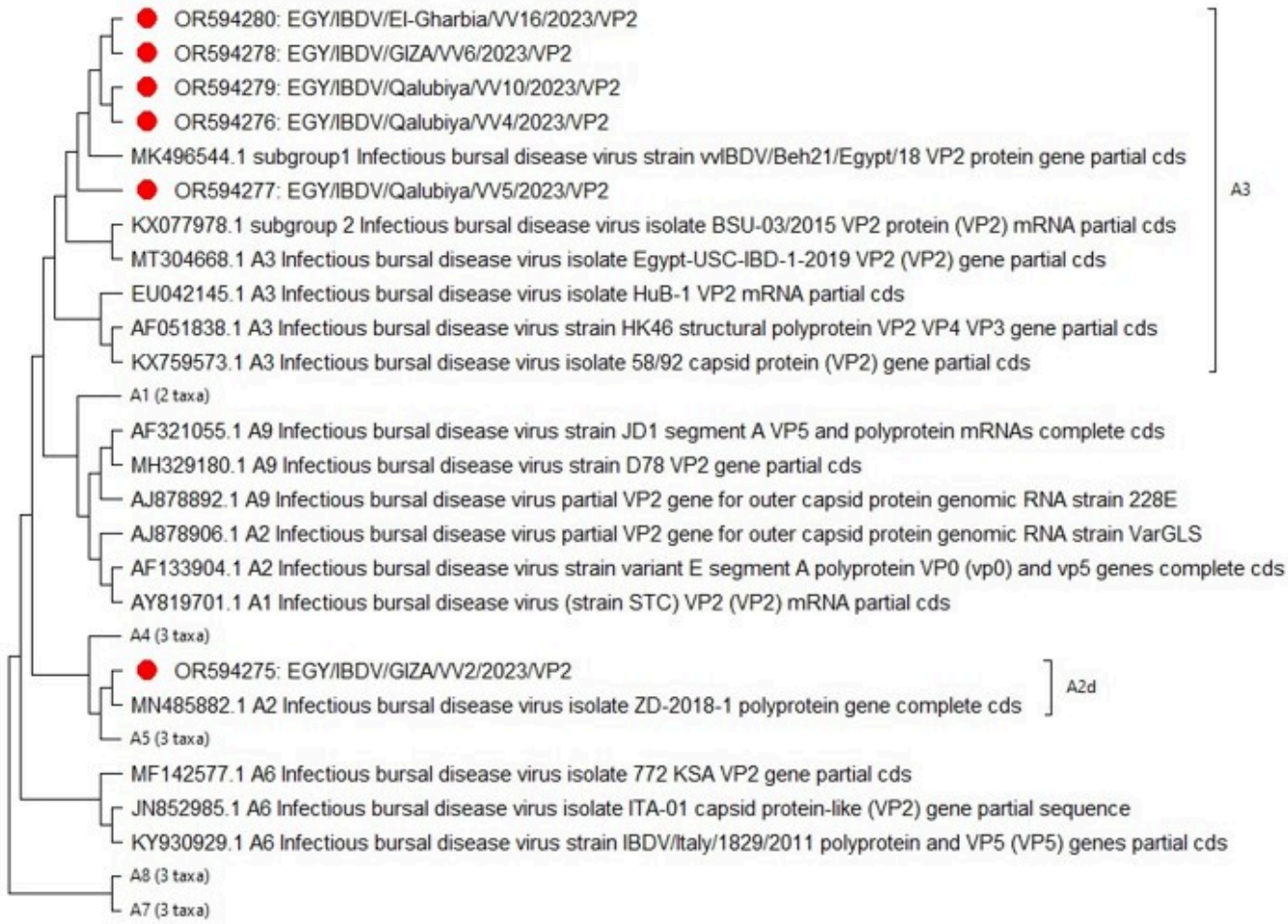


Figure 5

based on the nucleotide sequences by MAGE7.0. The phylogenetic tree represented the nucleotide sequences of VP2. The neighbour-joining phylogenetic trees were constructed using the Maximum Composite Likelihood model with 1000 bootstrap replicates.

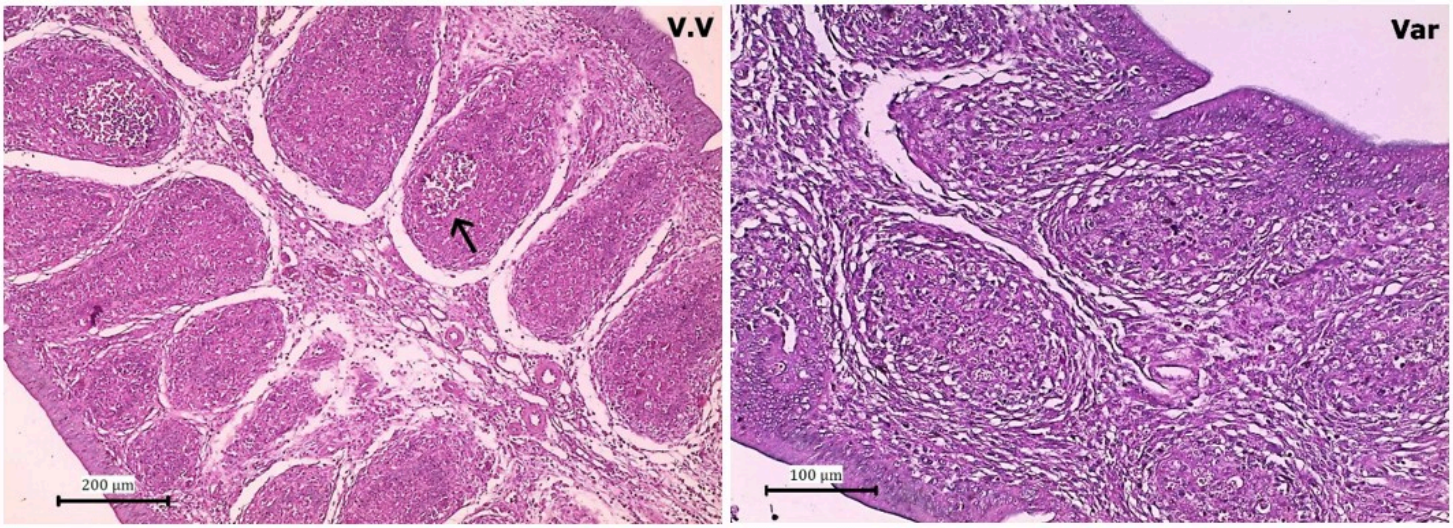


Figure 6

Photomicrograph of Bursa (VV): very virulent IBDV, bursa showed marked interlobular edema and lymphoid depletion (black arrow). (Var): Variant IBDV bursa showed massive lymphoid depletion with hyperplasia of bursal capsule.